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**400 Jahre**  
**UNIVERSITÄT GIESSEN**  
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**TEST REPORT**  
**VIRUCIDAL ACTIVITY AGAINST NOROVIRUSES**  
(under obligatory conditions according to the EN 14476 using feline calicivirus)

**a) Test laboratory**

Virology Laboratory of Clinic for Birds, Reptiles, Amphibian and Fish, Justus Liebig University Giessen

**b) Identification of the sample**

- 1) Name of the product: DISIFIN MED
- 2) Disinfectant sample: Tablets each of ~2.78 g
- 3) Batch number: 040108
- 4) Date of production: 2008-01
- 5) Date of delivery of the product: 30/09/2008

**6) Date of expiry:** 2011-01

**7) Manufacturer:** RMP chem. –techn. Spezialprodukte GmbH & Co. KG, 72119 Ammerbuch, Germany

**8) Storage conditions:** At room temperature, away from humidity and day light

**9) Appearance of the product:** White, round tablets

**10) Active substances and their concentrations:** 2.50 g tosylchloramid-Na/ tablet  $\pm 5 \%$

### **c) Experimental conditions**

**1) Period of testing:** 15/10/2008 – 17/11/2008

**2) Test temperature:**  $20 \pm 1 \text{ }^\circ\text{C}$

**3) Product diluent recommended by the manufacturer for use:** Water

**4) Method of Titration:** Quantal tests, dilution in  $\lg_{10}$  steps, virus titration using monolayers of cells on microtiter plates

**5) Concentrations of the product tested:** 0.05 %, 0.1 %, 0.25 %, 0.5 %, 1.0 %

**6) Contact times:** 60 min  $\pm 10$  s (obligatory)

**7) Procedure to stop action of product:** Dilution in ice-cold cell culture medium immediately at the end of the contact time

**8) Interfering substances:**

0.3 g/l bovine albumin (clean conditions), 3 g/l bovine albumin + 3 ml/l packed sheep erythrocytes in reaction mixture (dirty conditions)

**9) Product diluent:** Hard water

**10) Test-organism:** Feline calicivirus (FCV) strain F9

**12) Cell culture:** Crandell-Reese feline kidney (CRFK) cell line

**13) Incubation temperature:**  $37 \text{ }^\circ\text{C}$

### **d) Validation of test results**

**1) Titre of virus suspension:** 7.0-7.1 TCID<sub>50</sub>/ml

**2) Maximum detectable virus inactivation:**  $> 4 \lg$

### 3) Virus inactivation of the reference virus inactivation test: Not done

#### e) Presentation of the results

**1) Description of results:** In the first test, the virus titre of the control for clean conditions was 7.0 TCID<sub>50</sub>/ml. The control for dirty conditions had a titre of 7.1 TCID<sub>50</sub>/ml. In the second set, a titre of 7.1 TCID<sub>50</sub>/ml was determined for both control.

The results of cytotoxicity tests showed that the product has not cytotoxic effect within used dilution steps (level of cytotoxicity = <2.5).

The application of the dilution step 10<sup>-2</sup> of 0.5 % DISIFIN MED (lowest apparently non-cytotoxic dilution of the highest test concentration) on the cells for 60 min did not affect the virus susceptibility of the cell culture.

The 0.05 % and higher concentrations of DISIFIN MED led to disinfection of feline calicivirus under clean conditions. Under dirty conditions, the concentration of 0.05 % was ineffective. 0.1 % of DISIFIN MED disinfected the test virus only in the second test. However, the concentration of 0.25 % inactivated it in both tests.

#### 2) Tables of results

Table 1: Results of the tests under clean conditions

Product	Concentration of the active compound	Interfering substance	Level of cytotoxicity	Test Nr.	lg ID <sub>50</sub> /ml after 60 min	>4 lg reduction after ... min
DISIFIN MED	0.05 % (w/v)	0.3 g/l BSA	<2.5	1	<2.5	60
				2	<2.5	60
	0,1 % (w/v)		<2.5	1	<2.5	60
				2	<2.5	60
	0.25 % (w/v)		<2.5	1	<2.5	60
				2	<2.5	60
	0.5 % (w/v)		<2.5	1	<2.5	60
				2	<2.5	60
Virus control	n. a.	0.3 g/l BSA	n. a.	1	7.0	n. a.
				2	7.1	n. a.
Virus control of sensibility test	10 <sup>-2</sup> of 0.5 % (w/v)		n. a.	1	7.0	n. a.

n. a. not applicable  
n. d. not done

Table 2: Results of the tests under dirty conditions

Product	Concentration of the active compound	Interfering substance	Level of cytotoxicity	Test Nr.	lg ID <sub>50</sub> /ml after 60 min	>4 lg reduction after ... min	
DISIFIN MED	0.05 % (w/v)	3 g/l BSA + 3 ml/l erythrocytes	<2.5	1	5.9	-	
				2	5.1	-	
	0.1 % (w/v)		<2.5	1	3.4	-	
				2	<2.5	60	
	0.25 % (w/v)		<2.5	1	<2.5	60	
				2	<2.5	60	
	0.5 % (w/v)		<2.5	1	<2.5	60	
				2	<2.5	60	
	Virus control		n. a.	n. a.	1	7.1	n. a.
					2	7.1	n. a.
Virus control of sensibility test	10 <sup>-2</sup> of 0.5 % (v/v)	n. a.	n. a.	1	6.9	n. a.	
n. a. not applicable							
n. d. not done							

Table 3: Raw data of the product DISIFIN MED with 0.3 g/l BSA tested against feline calicivirus strain L9 (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Test Nr.	Dilutions (lg) <sup>a</sup>							
					2	3	4	5	6	7	8	
DISIFIN MED	0.05 % (w/v)	0.3 g/l BSA	60 min	1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
	0.1 % (w/v)			1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
	0.25 % (w/v)			1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
	0.5 % (w/v)			1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
DISIFIN MED Cytotoxicity test	0.05 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
	0.1 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
	0.25 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
	0.5 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
Virus control	n. a.	1	4444 4444	4444 4444	4444 4444	4444 4444	0400 0444	0000 0000	0000 0000			
		2	4444 4444	4444 4444	4444 4444	4444 4444	4400 4440	0000 0000	0000 0000			
a)		virus present, degree of CPE in 8 cell culture units (in wells of microtiter plates)										
1 to 4		no virus present										
0		not applicable										
n. a.		not done										
n. d.		not done										

Table 4: Raw data of the product DISIFIN MED with 3 g/l BSA + 3 ml/l erythrocytes tested against feline calicivirus strain L9 (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Test Nr.	Dilutions (lg) <sup>a</sup>							
					2	3	4	5	6	7	8	
DISIFIN MED	0.05 % (w/v)	3 g/l BSA + 3 ml/l erythrocytes	60 min	1	4444	4444	4444	0440	0000	0000	0000	
					4444	4444	4444	0000	0400	0000	0000	
	2			4444	4444	0000	4000	0000	0000	0000		
				4444	4444	4344	0000	0400	0000	0000		
	0,1 % (w/v)			1	0444	0000	0000	0000	0000	0000		
					4444	0000	0000	0000	0000	0000		
	2			0000	0000	0000	0000	n. d.	n. d.	n. d.		
				0000	0000	0000	0000	n. d.	n. d.	n. d.		
	0.25 % (w/v)			1	0000	0000	0000	0000	0000	0000		
					0000	0000	0000	0000	0000	0000		
	2			0000	0000	0000	0000	n. d.	n. d.	n. d.		
				0000	0000	0000	0000	n. d.	n. d.	n. d.		
0.5 % (w/v)	1	0000	0000	0000	0000	0000	0000					
		0000	0000	0000	0000	0000	0000					
2	0000	0000	0000	0000	n. d.	n. d.	n. d.					
	0000	0000	0000	0000	n. d.	n. d.	n. d.					
DISIFIN MED Cytotoxicity test	0.05 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
	0.1 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
	0.25 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
	0.5 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
Virus control	n. a.	1	4444	4444	4444	4444	4044	0000	0000			
			4444	4444	4444	4444	0404	0000	0000			
2	4444	4444	4444	4444	4440	0000	0000					
	4444	4444	4444	4444	0404	0000	0000					
a) 1 to 4 virus present, degree of CPE in 8 cell culture units (in wells of microtiter plates) 0 no virus present n. a. not applicable n. d. not done												

Table 5: Results of the tests carried out for determination of cell sensitivity to virus.

Product	Concentration	Interfering substance	Dilution step (10 <sup>1</sup> )	Dilutions (lg) <sup>a</sup>							
				2	3	4	5	6	7	8	
DISIFIN MED Cytotoxicity test	0.5 % (w/v)	0,3 g/l BSA	2	4444	4444	4444	4444	0004	0000	0000	
				4444	4444	4444	4444	0444	0000	0000	
		3 g/l BSA + 3 ml/l erythrocytes		4444	4444	4444	4444	0440	0000	0000	
				4444	4444	4444	4444	0040	0000	0000	
a) 1 to 4 virus present, degree of CPE in 8 cell culture units (in wells of microtiter plates) 0 no virus present n. d. not done											

### 3) Graphic presentation of all results

Figure 1: Graphic presentation of results (clean conditions, see table 1)

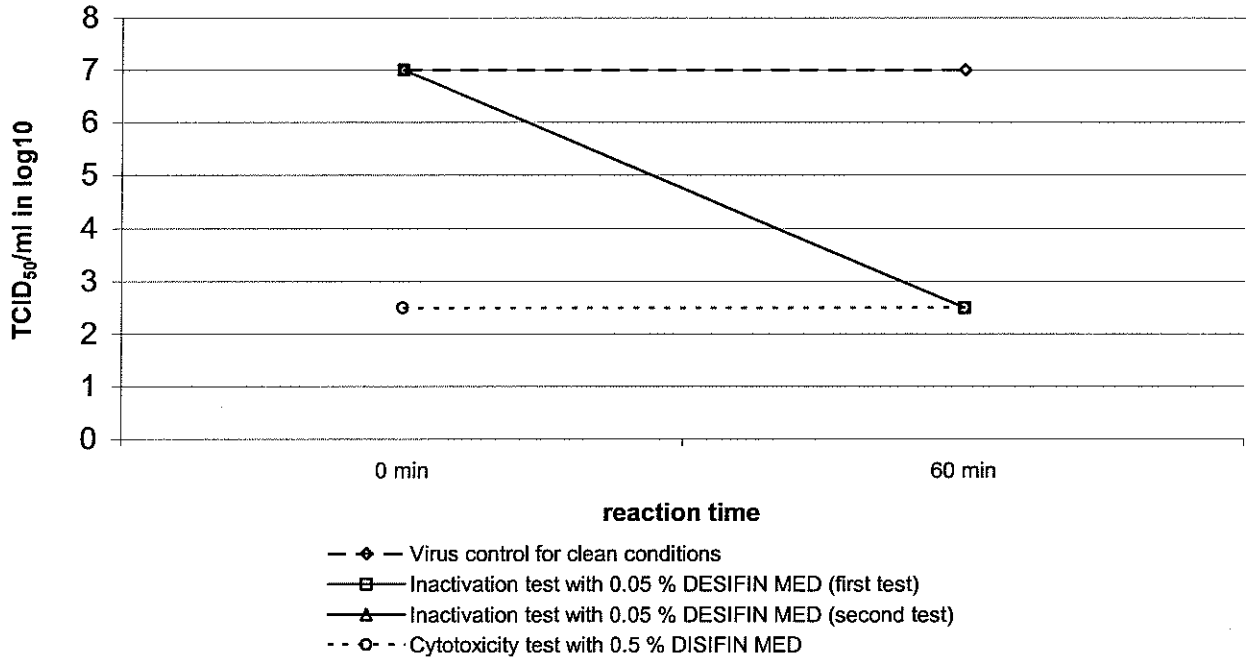
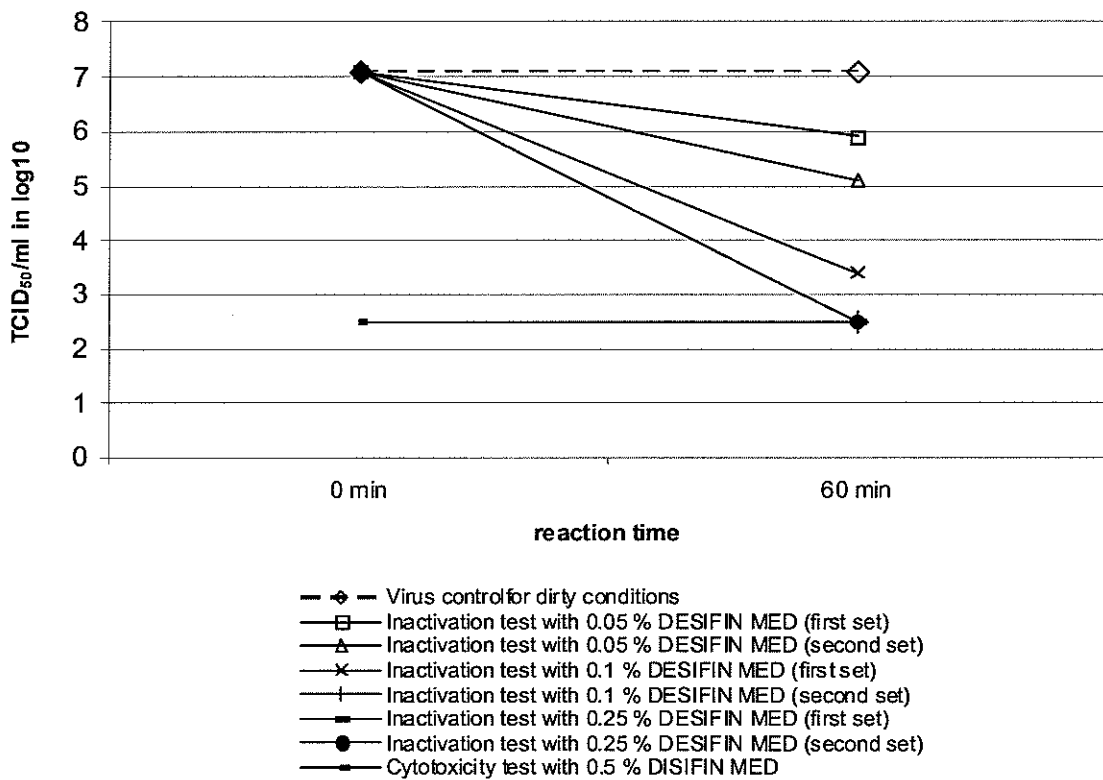


Figure 2: Graphic presentation of results (dirty conditions, see table 2)



**f) Conclusion:** For the product DISIFIN MED, the virucidal concentration against feline calicivirus determined according to the EN 14476 (Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine – Test method and requirements (phase 2, step 1). Date February 2007) at obligatory conditions (test temperature:  $20 \pm 1$  °C; contact time: 60 min) is:

**0.05 % or higher under clean conditions**

**0.25 % or higher under dirty conditions**

Because the structure of viruses within a virus family is very similar it is assumed that the results of these examinations are also applicable for disinfections of other caliciviruses including member of the genus Norovirus (Norwalk-like viruses).

17/11/2008



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